

Introduction of 2,4-dichlorophenoxyacetic acid into soil with solvents and resulting implications for bioavailability to microorganisms

Teresa A. Johnson · Gerald K. Sims

Received: 23 June 2010/Accepted: 3 September 2010/Published online: 18 September 2010
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Abstract Slow equilibration of introduced chemicals through tortuous pore space limits uniform substrate distribution in soil biodegradation studies. The necessity of introducing poorly soluble xenobiotics via organic solvents, the volume of which is minimized to limit toxicity, likely also affects xenobiotic distribution. Our objective was to investigate relative effects of carrier solvent choice and volume on xenobiotic distribution, apparent solvent toxicity, and soil degradation of 2,4-dichlorophenoxy acetic acid. Incubations using four carrier solvents ranging in properties showed that the fraction of 2,4-D mineralized was a hyperbolic function of solvent volume used ($0.02\text{--}10 \mu\text{l g}^{-1}$), attributed to compensating effects of herbicide bioavailability and solvent toxicity. Substrate concentration influenced mineralization of herbicide introduced with organic carriers, but not water. Fraction of material readily desorbed increased when water was the carrier. Results suggest that solvent toxicity should be balanced with uniformity of substrate distribution when using organic carriers in soils. Substrate bioavailability is a ubiquitous issue in terrestrial microbiology research, thus limitations observed herein broadly apply to microbiology questions about introduced substances in soil. We advocate the development of tools to characterize variable conditions among soil compartments, estimates of substrate

bioavailability, and linkage of this information to microbial data.

Keywords Biodegradation · Herbicide · Pesticide · Sorption · Desorption · Mineralization

Introduction

Degradation of xenobiotics in soil is influenced by soil physical and chemical properties, as well as organisms present, and thus can exhibit considerable variability across soils when experimental conditions are kept constant (Picton and Farenhorst 2004; Sims and Cupples 1999). Moreover, degradation has been shown to vary as a function of both the mechanical and chemical aspects of introduction and distribution methods (Shelton and Parkin 1989; Parkin et al. 1991; Skidmore et al. 1994). Choice of spiking solvent and initial soil water content can also have profound effects on extraction recovery, as demonstrated with the herbicide, 2,4-dichlorophenoxy acetic acid (Merini et al. 2008). By far, the most common approach used to date has been introduction of xenobiotics as relatively large droplets with a syringe or pipet (Lehmann et al. 1993; Mervosh et al. 1995a), though numerous approaches and variations have been employed or proposed. Shelton and Parkin (1989) and Parkin et al. (1991) compared various introduction methods, such as atomization of the substrate onto sieved soil, application to a soil slurry, and injection of undisturbed soil cores. Generally, the techniques able to reach the largest number of surfaces initially have also introduced the greatest soil disturbance, and in the case of application as an atomized spray, introduced uncertainty in uniformity of loading rates among samples, owing to a significant portion of material that miss the target (also increasing risk of lab

T. A. Johnson
University Center for Advanced Teaching, The Ohio State University, Columbus, OH 43201, USA

G. K. Sims (✉)
Global Change and Photosynthesis Research Unit, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), S-306 Turner Hall, 1102 S. Goodwin Ave, Urbana, IL 61801, USA
e-mail: gksims@illinois.edu

contamination in the case of radioisotopes). Nonetheless, atomization is effective for improving uniformity, and has been especially useful for studies in which substrates are rapidly transformed in the presence of water (Taylor-Lovell et al. 2002). Skidmore et al. (1994) concluded that homogeneity of permethrin distribution (as influenced by application method) significantly affected degradation kinetics. Sims (2008) observed that more than 100 h may be required for redistribution of a radio-labeled substance added to 15 aggregates to become detectable in 35 adjacent aggregates when 30% of the pore space is filled with water, suggesting that much of the microbial population would not be exposed to an introduced substance for at least several days.

The choice of a carrier solvent for an organic compound is usually based on the aqueous solubility (and stability) of the test substance and desired soil moisture content (Hixson et al. 2009; Taylor-Lovell et al. 2002). When the volume of water required to dissolve the test substance exceeds targeted soil moisture content, an organic delivery solvent is used instead (Chakraborty et al. 1995; Kruger et al. 1997; Reimer et al. 2005), typically at a lesser volume than the desired water content. Merini et al. (2008) reported greater initial recovery of 2,4-D applied with methanol than with water, and that recovery increased continuously with methanol volume, requiring 168 µl/g to achieve maximal recovery (resulting in a soil solution phase containing >65% methanol). Alcohols at or near this concentration are employed widely as surface sterilants, and would likely suppress microbial degradation. Organic solvents generally can be toxic to, or promote growth of soil microorganisms (Buddin 1914; Satsuma et al. 2001; Tor et al. 2000), are variable in chemical properties, and are poorly described as delivery systems with respect to uniformity of distribution of xenobiotics in soil. Extensive mixing or reducing aggregate size through grinding and sieving are commonly used to improve substrate distribution, but impose dramatic disturbance of soil structure, which may alter transport of microbial substrates (including oxygen). Since compartmentalization due to tortuous pore space is likely responsible for both the great diversity and functional redundancy of unsaturated soils (Zhou et al. 2004), preservation of structure is highly desirable when studying microbial processes in soils if detection of diverse populations involved in the process is a goal. Improved methods for detection of microorganisms in environmental samples and especially those methods that link function with phylogeny can now facilitate identification of organisms responsible for specific soil processes, such as biodegradation. For this purpose, however, such techniques rely upon the homogeneous introduction into soil of a test substance (Cupples and Sims 2007; Sims 2008), often isotopically labeled, though the degree to which introduction methods achieve this goal is seldom characterized.

Among herbicides, atrazine (Radosevich and Tuovinen 2004) and 2,4-D (Zaprasis et al. 2010) have received, by far, the greatest attention in development of molecular ecology tools, and thus have become the primary models for studying microbial ecology of herbicide degradation. The objective of this work was to investigate the relative effects of carrier solvent choice and volume on apparent solvent toxicity, substrate bioavailability, and herbicide degradation rates in a soil. The herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), was chosen, owing to its widespread use and central role as a model compound for examining the microbial ecology of xenobiotic degradation. Moreover, 2,4-D is relatively soluble, minimally sorbed in soils, and would be expected to redistribute more rapidly than hydrophobic substrates, thus it can serve as a conservative indicator of the severity of artifacts related to bioavailability. The herbicide was introduced into the soil using two polar protic solvents (water and methanol), a polar aprotic solvent (acetone) and a nonpolar aprotic solvent (ethyl acetate), to provide a variety of solvent properties with potential to influence soil distribution.

Materials and methods

Chemicals

Radiolabeled 2,4-D [UL^{-14}C] (4×10^{10} Bq mmol $^{-1}$) was purchased from Sigma Chemical Co., St. Louis, MO. All solvents and water used were OptimaTM, acquired from Fisher Scientific (Pittsburgh, PA). Table 1 summarizes relevant properties of the delivery solvents, including boiling point, density, solubility and Henry's Law Constant (Lide 2002), 2,4-D solubility (Vencill 2002), and Hansen Solubility Parameters (Hansen 2007). The scintillation cocktail, Bio-Safe IITM, was purchased through Research Products International Corp. (Mount Prospect, IL). All other chemicals used were reagent grade and were obtained from Fisher Scientific or Sigma Chemical Co.

Soil

Thorp silt loam (fine-silty, mixed, mesic Argiaquic Argialbolls) was sampled from the top 15 cm from a site in Urbana, IL. The Thorp soil has a very fine, friable, granular structure of moderate strength, and was sieved to recover 0.4–2 mm size aggregates (typical surface aggregate size range for this soil) with the minimal disturbance achievable. Samples were either air-dried (extraction/recovery study) or stored at field moisture in sealed polyethylene bags at 4°C until use (degradation and desorption studies). Properties of the Thorp soil are listed in Table 2.

Table 1 Properties of solvents used for delivery of 2,4-D

| Solvent | Boiling point [†] (°C) | Density [†] (g cm ⁻³) | Solubility in water [†] (g l ⁻¹) | Henry's law constant [†] (mol _{aq} /m ³ _{aq}) Pa ⁻¹ | Solubility of 2,4-D (g l ⁻¹) | Hansen solubility parameters [¶] | | |
|---------------|------------------------------------|---|--|--|--|---|-------------------------------------|-------------------------------------|
| | | | | | | δD (MPa ^{0.5}) | δP (MPa ^{0.5}) | δH (MPa ^{0.5}) |
| Water | 100 | 1.00 | Miscible | | 0.90 [‡] | 15.5 | 16.0 | 42.3 |
| Methanol | 65 | 0.79 | Miscible | 2.171 | 1100 [§] | 14.7 | 12.3 | 22.3 |
| Acetone | 56 | 0.78 | Miscible | 0.296 | 670 [‡] | 15.5 | 10.4 | 7.0 |
| Ethyl acetate | 77 | 0.89 | 83 | 0.058 | 191 [§] | 15.8 | 5.3 | 7.2 |

[†] From Lide (2002)[‡] From Vencill (2002)[§] Estimated by LSS of <0.2 µm filtrate following equilibration of ¹⁴C-labeled 2,4-D with solvent at 20°C for 1 week[¶] From Hansen (2007)**Table 2** Properties of Thorp silty clay loam soil

| Select chemical properties | | | | Exchangeable nutrients [†] (µg g ⁻¹ soil) | | | | Moisture release curve [‡] (% water) | | | | |
|----------------------------------|-----|------------------------|----------------------------------|--|----|-----|-----|---|---------|---------|----------|----------|
| OC (µg kg ⁻¹ soil) | pH | Clay content (%) | CEC (cmolc kg ⁻¹) | K _d 2,4-D acia (l kg ⁻¹) | P | K | Mg | Ca | -10 kPa | -33 kPa | -100 kPa | -700 kPa |
| 2.47 | 6.1 | 57 | 14.0 | 0.49 | 88 | 420 | 235 | 1800 | 30.8 | 26.2 | 20.0 | 11.9 |

OC organic carbon (w/w), CEC cation exchange capacity, K_d equilibrium batch sorption isotherm[†] Analyses performed by A&L Laboratories, Ft. Wayne, IN[‡] Characteristic water retention curve: -kPa = 9261.6 e^{(-0.2202)%H₂O}

The characteristic water retention curve was performed as described by Dane et al. (2002), effective cation exchange capacity (CEC) was determined by sum of cations (Sumner and Miller 1996), and equilibrium batch sorption isotherms (soil/solution = 1/5) were performed according to Mervosh et al. (1995b). Soil mass is reported herein on a dry weight basis unless otherwise specified.

Incubation conditions

Field moist soil (15–50 g) was placed in mason jar biometers fitted with CO₂ traps containing 2 ml of 0.2 M NaOH (Mervosh et al. 1995a). Water was added to three-fourths of the biometers used in the degradation study such that the final pressure was approximately -180 kPa (17.9% H₂O w/v). The volume of water used for the remaining biometers was reduced to compensate for subsequent addition of water as a carrier solvent. Biometers were then sealed and incubated at 25°C for 48 h to allow soil to equilibrate with the added water. Soils used in the extraction recovery study were initially air-dry. Moisture content was adjusted to -180 kPa as described above (resulting in the same final moisture regime across experimental treatments), with an additional air-dry treatment that received no added water.

Extraction recovery study

Methanol was used as a carrier solvent and was introduced at rates of 1 and 10 µl g⁻¹ soil. After addition of the solvent, the soil was subjected to minimal mixing. Analysis consisted of removing eighteen 2.5-g subsamples for extraction with 5 ml methanol and quantification of extracted radioactivity with LSS. Treatments were evaluated for coefficients of variation in recovered radioactivity. Statistical analyses consisted of analysis of variance using the SAS system (SAS Institute, Cary, NC). The class variable was solvent volume.

Degradation study

After a 48-h equilibration period, a range of volumes (0.02–10 µl g⁻¹ soil) of water, methanol, ethyl acetate, or acetone was used to incorporate 3,450 Bq 2,4-D [UL-¹⁴C] which had been diluted with unlabeled material to produce a final soil concentration of either 0.1 or 1 µg 2,4-D g⁻¹ soil, which would produce maximum solution concentrations of 0.6 or 6.0 µg 2,4-D ml⁻¹, assuming no sorption, and thus would be expected to be well below reported thresholds (25–50 µg ml⁻¹) for toxicity of 2,4-D or its metabolites to degraders (Shaw et al. 2000). Minimum

volume for aqueous addition was $0.04 \mu\text{l H}_2\text{O g}^{-1}$ at a concentration of $0.1 \mu\text{g 2,4-D g}^{-1}$ soil and $1 \mu\text{l H}_2\text{O g}^{-1}$ at $1 \mu\text{g 2,4-D g}^{-1}$ soil due to solubility limitation for the test substance (Table 1). Biometers were incubated at 25°C , and non-destructively sampled repeatedly up to 35 days after application of the test substance. In order to ensure proper aeration, each biometer was opened every 3–4 days and the CO_2 traps were exchanged. At each sampling time, contents of CO_2 traps from the degradation study were transferred to scintillation vials, mixed with scintillation cocktail, and radioactivity was quantified with liquid scintillation spectrometry (LSS) using a 1600 TR Liquid Scintillation Analyzer (Packard, Downers Grove, IL).

Desorption of aged 2,4-D

Desorption of 2,4-D was measured in Thorp soil treated with ^{14}C -labeled 2,4-D as described above and incubated for periods up to 60 days. The herbicide was applied to triplicate biometers with either ethyl acetate or water in a $10 \mu\text{l}$ volume as described above. At each incubation point, 1 g of soil from each replicate was weighed into a separate 8-ml scintillation vial to which 5 ml of 0.01 M CaCl_2 was added immediately followed by agitation as described by Mervosh et al. (1995b) for either 15 min or 24 h. After agitation, a 1.5-ml aliquot was transferred to a microcentrifuge tube and centrifuged at $11,750 \text{ g}$ for 20 s. One milliliter of the supernatant was analyzed by LSS as described above.

Redistribution of 2,4-D by advection and capillary movement

Filter paper was used to simulate the porous nature of soil in order to establish potential differences between solvents in the nature of 2,4-D redistribution as the solvent front retreated by evaporation. ^{14}C -2,4-D (183 Bq) was applied to the center of duplicate strips ($3 \text{ mm} \times 40 \text{ mm}$) of filter paper (enclosed in PTFE film) in either ethyl acetate ($18 \mu\text{l}$) or water ($11 \mu\text{l}$) to wet the paper to within 3 mm of the termini. For each solvent, one strip remained covered in PTFE film and was sectioned into thirds 15 s after application of 2,4-D. The other strip was uncovered and allowed to dry, after which it was sectioned in 3 mm increments up to 15 mm from the point of application.

Results and discussion

Time-averaged coefficients of variation for extraction recovery from methanol-applied 2,4-D are depicted in Fig. 1. Herbicide distribution was more uniform in air-dry soil owing to more effective mixing in this soil (less

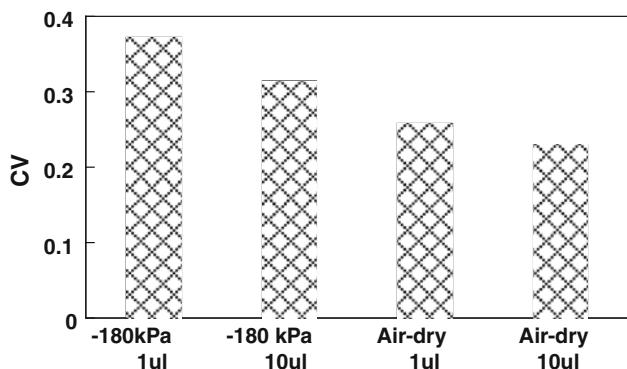


Fig. 1 Time-averaged coefficients of variation for methanol extraction recovery from methanol-applied 2,4-D. Data presented are for three replicates (each a mean of six sub-samples) averaged for all three sampling periods (1.5, 48, and 144 h)

clumping). Results also show that variability in recovery among replicates was less when the herbicide was introduced with more carrier solvent, which was attributed to enhanced uniformity of distribution. Based on observations of Merini et al. (2008), this pattern of increased recovery would be expected to continue even up to very large rates of carrier solvent (which would be expected to destroy most vegetative bacterial cells present). As expected, variability decreased over time (data not shown). It follows that in order to optimize distribution of test substance, solvent volume should be as large as can be achieved without affecting other parameters. In the case of 2,4-D, it is apparent that the upper limit for solvent volume (due to toxicity effects of solvents) will generally be well below the quantity needed for optimal recovery. 2,4-D is particularly mobile in soil (K_d for Thorp soil = 0.49 l kg^{-1}), with a diffusion coefficient in the range of $7 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ (Kim et al. 2008), which is one to seven orders of magnitude greater than that for atrazine (Walker and Crawford 1970) or trifluralin (Bode et al. 1973), respectively. Thus, it is likely that achieving a relatively uniform distribution will be a greater challenge for most herbicides than was observed 2,4-D here.

The fraction of applied 2,4-D mineralized as a function of carrier solvent volume is shown in Fig. 2. Regardless of experimental scale, application of 2,4-D in water resulted in an increase in mineralization with increase in carrier volume, although no further increase was observed using volumes greater than $2 \mu\text{l g}^{-1}$ soil. This observation is consistent with increased advective transport of material expected with a greater volume of added water, as apparently was the case for the extraction recovery study above performed with methanol here as well as results presented by Merini et al. (2008). This would be expected to provide a population of degraders with physical access to a greater number of pores containing herbicide, or similarly, exposing the herbicide to a larger population (or populations) of

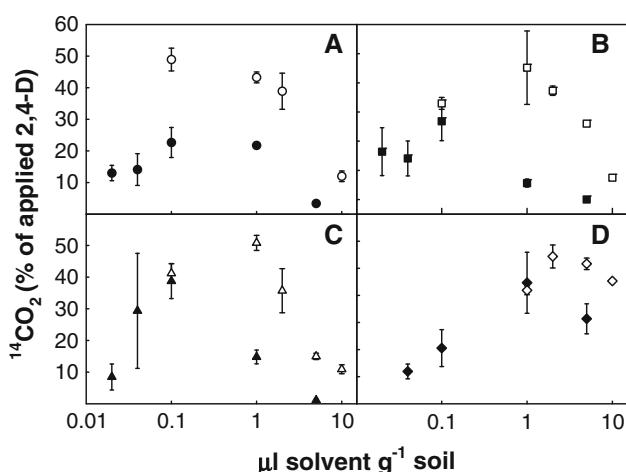


Fig. 2 The fraction of applied 2,4-D mineralized as a function of carrier solvent type and volume used: **a** methanol, **b** ethyl acetate, **c** acetone, **d** water. Data are means of three replicates. *Closed symbols* represent treatments receiving $0.1 \mu\text{g} 2,4\text{-D g}^{-1}$ soil, *open symbols* represent treatments receiving $1.0 \mu\text{g} 2,4\text{-D g}^{-1}$ soil

organisms capable of degrading 2,4-D. Gonod et al. (2003) observed that 2,4-D degraders were distributed unevenly when measured on scales less than one cm, and tended to aggregate in “hot spots”, which occurred at approximately a 1-cm scale. For water application, increases in distribution resulting from further increase in aqueous volume may have been offset by kinetic constraints induced by dilution of the substrate.

All of the organic carrier solvents exhibited a hyperbolic mineralization response with a peak at volumes of 0.1 or $1 \mu\text{l}$ carrier solvent g^{-1} soil. Based only on mineralization kinetics, it appears optimal solvent volume exhibits a relatively narrow range in this system. Each of the organic solvents used was relatively more volatile than water, thus it is expected that material advected into soil pore space would be deposited as a precipitate or dissolve into soil solution as the solvent evaporated. The existence or location of a precipitate within the pore space continuum cannot be ascertained from the data herein. However, each of the organic solvents likely retained a greater mass of material in solution than did water as the solvent front recedes (Table 1), and thus was likely to produce a different initial pattern of solute deposition than water. As noted in Table 1, the Henry’s Law constants for the organic solvents used here decreased with decreasing Hansen’s parameter for polarity (δP), indicating ethyl acetate would be expected to be volatilized from moist soil most rapidly, and have the greatest affinity for hydrophobic microsites as the solvent retreated.

Figure 3 reports the desorption of 2,4-D as a function of incubation time. Initially, most of the material was recovered within the first 15 min of agitation. Over the duration of the incubation, this pool diminished rapidly in both the

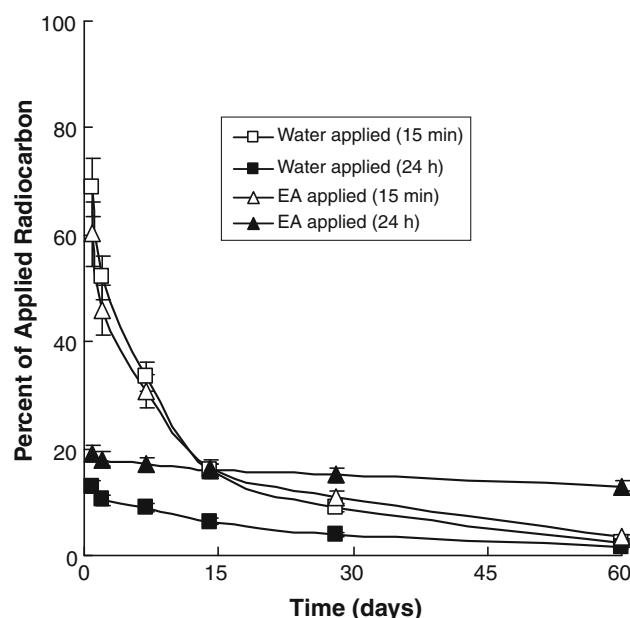


Fig. 3 Desorption of 2,4-D from Thorp soil as a function of carrier solvent and soil incubation time. *Open symbols* show 2,4-D desorbed in 15 min of agitation in 0.01 M CaCl_2 , whereas *closed symbols* show desorption after 24 h of agitation. *Triangles* represent treatments applied with ethyl acetate and *squares* indicate treatments applied with water

ethyl acetate and water applied treatments. The portion of material requiring up to 24 h of agitation for recovery also was depleted over time, but constituted a larger portion of the total later in the incubation period. This pool was depleted more slowly in the ethyl acetate treatment, suggesting a greater portion of the 2,4-D applied with ethyl acetate resided in sites exhibiting slower desorption kinetics (and likely limited bioavailability). Hermosín et al. (2006) observed that partitioning of 2,4-D into hydrophobic supports, such as organo-clays, slows release of the herbicide to solution and impedes degradation. Similarly, 2,4-D sorption increases with soil organic carbon content (Picton and Farenhorst, 2004; Farenhorst et al. 2008) and likewise bioavailability appears to limit degradation of the herbicide at the regional scale (Farenhorst et al. 2008). Thus, even for a highly soluble herbicide, such as 2,4-D, potential for bioavailability limitation must be considered when developing experimental protocols. Association with dissolved organic carbon (Shaw et al. 2000) can affect 2,4-D metabolite bioavailability, however the techniques used herein to measure desorption would not have distinguished free 2,4-D from that associated with soluble carbon.

Figure 4 shows the distribution of 2,4-D as a function of distance from the point of application to filter paper in either ethyl acetate or water. No significant difference was observed among sections when filter paper was cut wet (data not shown). However, in Fig. 4, it is evident that the organic solvent carried the bulk of the added material with

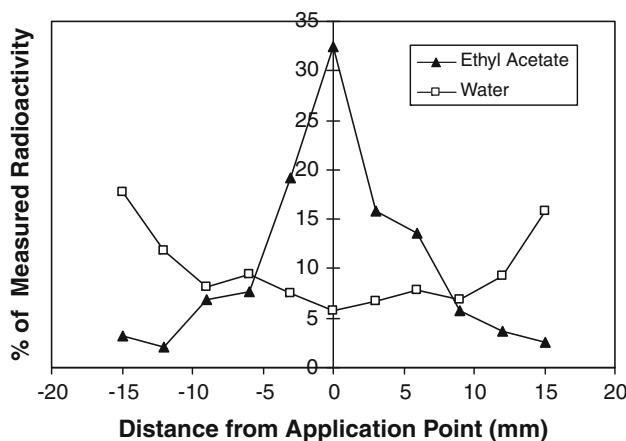


Fig. 4 Distribution of ^{14}C -2,4-D as a function of distance from the point of application to filter paper in either ethyl acetate or water. Triangles represent application with ethyl acetate and squares show the treatment applied with water

it as the solvent front retreated toward the center, whereas 2,4-D added in water was deposited along the solvent front as the water evaporated. In soil, it is expected that both organic solvents and water will retreat into small pore space as they evaporate, however, organic solvents will be lost much more rapidly than water. The results of Fig. 4 would suggest that a considerable portion of the deposited material may be concentrated in a relatively small portion of the pore space when a hydrophobic organic solvent is used. This effect would be expected to vary among solvents as the substrate solubility (and affinity for the solid phase), capillary rise, and volatility of the carrier solvent varies. It is also possible that the nature of deposition sites may vary with solvent properties.

Owing to a relatively low pKa value of 2.8 (Vencill 2002), 2,4-D was likely completely dissociated in the Thorp soil ($\text{pH} = 6.1$). The aprotic solvents (acetone and ethyl acetate), which differ primarily in polarity (δP) among the Hansen solubility parameters (HSP) presented in Table 1, produced similar 2,4-D mineralization responses. Though HSP data were not available for 2,4-D, aromatic carboxylic acids generally exhibit δP values intermediate between acetone and ethyl acetate, whereas hydrogen bonding (δH) values for these compounds tend to be intermediate between acetone and methanol (Hansen 2007). This likely explains why methanol was the best solvent for 2,4-D.

Decreases in mineralization at solvent volumes greater than optimum were attributed primarily to toxicity in the case of organic solvents. Miscible organic solvents as well as immiscible solvents with $\log P$ ($P = \text{octanol/water partitioning coefficient}$) values <2 are generally not tolerated by bacteria, owing to a greater exposure of the cell aqueous phase (Matsumoto et al., 2004). Ethyl acetate was the only non-miscible solvent examined herein, but is quite soluble,

with a $\log P$ value of 0.7 (Jorba et al., 1995), thus all of these organic solvents would be expected to exert at least a transient toxic effect on most bacteria present. Skidmore et al. (1994) reported toxicity effects from common laboratory solvents applied at rates of $1\text{--}13 \mu\text{l g}^{-1}$ soil (producing solution concentrations in the range of $4\text{--}52 \times 10^3 \mu\text{g ml}^{-1}$), though solvents range widely in toxicity, as does sensitivity to solvents among the diverse groups of microorganisms represented in soils. The rates of miscible organic solvents used in this study were quite low compared to the bulk of the literature, but still should have produced soil solution concentrations approximately $1\text{--}50 \times 10^3 \mu\text{g ml}^{-1}$. In addition to potential toxicity, this would have provided up to four orders of magnitude more carbon as solvent than as 2,4-D, which is also an important consideration for biodegradation studies.

Because of the toxicity effect observed with many organic solvents, it is preferable to use water as a carrier whenever possible. Based on results herein, it also appears that distribution within pore space may be more uniform when water is used, though 2,4-D may not be representative of more hydrophobic compounds. When organic solvents must be used, both toxicity and extent of distribution should be empirically optimized (rather than simply minimizing carrier volume). Including an indicator of the accessibility of the soluble substrate pool (e.g. residual substrate persisting in soil solution) may assist in interpreting results since a significant fraction of treated pore space may not be inhabited by degraders (Gonod et al. 2003; Johnson et al. 1998). After application, further distribution of the added material over time will depend on factors such as retention by sorption to solids and the portion of pore space filled with water (which defines cross sectional area available for diffusion). These results indicate that considerable variability in outcome may occur with different xenobiotic application procedures. This is particularly important for an approach such as stable isotope probing, which exhibits its best performance (in terms of selectivity against artifacts arising from cross-feeding) early in the incubation, before the test substance has diffused thoroughly through the soil matrix (Sims 2008). Moreover, substrate bioavailability is broadly relevant to terrestrial microbiology research. Compartmentalization due to tortuous pore space is likely responsible for both the great diversity and functional redundancy of unsaturated soils. Such compartmentalization also means that it is difficult to simultaneously expose (either naturally or experimentally) all of this discontiguous space (and organisms therein) to the same substance. Moreover, the physicochemical environments among soil compartments likely vary profoundly, and the ability to readily measure (and link to microbial data) the pore environment would greatly empower a researcher attempting to interpret molecular microbiology data from soil.

Acknowledgments The authors appreciate the assistance of Christina Hüneke for laboratory assistance. This work was supported by the Agricultural Research Service, United States Department of Agriculture, project number 3611-12220-006-00D. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

References

- Bode LE, Day CL, Gebhardt MR, Goering CE (1973) Prediction of trifluralin diffusion coefficients. *Weed Sci* 21(5):485–489
- Buddin W (1914) Partial sterilization of soil by volatile and non-volatile antisepsics. *J Agric Sci* 6:417–455
- Chakraborty SK, Chowdhury A, Bhattacharyya A (1995) Microbial degradation of oxadiazon by soil fungus *Fusarium solani*. *J Agric Food Chem* 43:2964–2969
- Cupples AM, Sims GK (2007) Identification of in situ 2, 4-dichlorophenoxyacetic acid-degrading soil microorganisms using DNA-stable isotope probing. *Soil Biol Biochem* 39:232–238
- Dane JH, Hopmans JW, Romano N, Nimmo J, Winfield KA (2002) Soil water retention and storage—laboratory methods. In: Dane JH, Topp GC (eds) *Methods of soil analysis part 4 physical methods*. Soil Science Society of America, Madison, pp 675–720
- Farenhorst A, Londry KL, Nahar N, Gaultier J (2008) In-field variation in 2, 4-D mineralization in relation to sorption and soil microbial communities. *J Environ Sci Health B* 43(2):113–119
- Gonod LV, Chenu C, Soulas G (2003) Spatial variability of 2, 4-dichlorophenoxyacetic acid (2, 4-D) mineralization potential at a millimetre scale in soil. *Soil Biol Biochem* 35:373–382
- Hansen CM (2007) Hansen solubility parameters: a user's handbook, 2nd edn. CRC Press Taylor Francis Group, Boca Raton 544 p
- Hermosín MC, Celis R, Carrizosa MJ, Ortega-Calvo JJ, Cornejo J (2006) Bioavailability of the herbicide 2, 4-D formulated with organoclays. *Soil Biol Biochem* 38(8):2117–2124
- Hixson AC, Wei S, Weber JB, Yelverton FH, Rufty TW (2009) Soil organic matter changes in turfgrass systems affect binding and biodegradation of simazine. *Crop Sci* 49:1481–1488
- Johnson TA, Sims GK, Ellsworth TR, Balance AM (1998) Effects of moisture and sorption on biodegradation of p-hydroxybenzoic acid by *Arthrobacter* sp. *Microbiol Res* 153:349–353
- Jorba X, Clapés P, Torres JL, Valencia G, Mata-Alvarez J (1995) Ethyl acetate modified AOT water-in-oil microemulsions for the α -chymotrypsin catalyzed synthesis of a model dipeptide derivative. *Colloids Surf A Physicochem Eng Asp* 96(1–2):47–52
- Kim T-Y, Park SS, Kim SJ, Cho S-Y (2008) Separation characteristics of some phenoxy herbicides from aqueous solution. *Adsorption* 14:611–619
- Kruger EL, Rice PJ, Anhalt JC (1997) Comparative fates of atrazine and deethylatrazine in sterile and nonsterile soils. *J Environ Qual* 26:95–101
- Lehmann RG, Fontaine DD, Olberding EL (1993) Soil degradation of flumetsulam at different temperatures in the laboratory and field. *Weed Res* 33:187–195
- Lide DR (2002) CRC handbook of chemistry and physics, 83rd edn. CRC Press Taylor Francis Group, Boca Raton 2664 p
- Matsumoto M, Mochiduki K, Kondo K (2004) Toxicity of ionic liquids and organic solvents to lactic acid-producing bacteria. *J Biosci Bioeng* 98(5):344–347
- Merini LJ, Cuadrado V, Giulietti AM (2008) Spiking solvent, humidity and their impact on 2, 4-D and 2, 4-DCP extractability from high humic matter content soils. *Chemosphere* 71(11): 2168–2172
- Mervosh TL, Sims GK, Stoller EW (1995a) Clomazone fate in soil as affected by microbial activity, temperature, and soil moisture. *J Agric Food Chem* 43:537–543
- Mervosh TL, Sims GK, Stoller EW, Ellsworth TR (1995b) Clomazone sorption in soil: incubation time, temperature, and soil moisture effects. *J Agric Food Chem* 43:2295–2300
- Parkin TB, Shelton DR, Robinson JA (1991) Evaluation of methods for characterizing carbofuran hydrolysis in soil. *J Environ Qual* 20:763–769
- Picton P, Farenhorst A (2004) Factors influencing 2, 4-D sorption and mineralization in soil. *J Environ Sci Health B* 39(3):367–379
- Radosevich M, Tuovinen OH (2004) Microbial degradation of atrazine in soils, sediments, and surface water. In: *Pesticide decontamination and detoxification*. ACS Symposium Series, vol 863. American Chemical Society, pp 129–139
- Reimer M, Farenhorst A, Gaultier J (2005) Effect of manure on glyphosate and trifluralin mineralization in soil. *J Environ Sci Health B* 40:605–617
- Satsuma K, Nakamura H, Sato K, Kato Y (2001) A negative effect of co-solvent on atrazine biodegradation in experimental river microcosms. *Microbes Environ* 16(3):185–189
- Shaw LJ, Beaton Y, Glover LA, Killham K, Osborn D, Meharg AA (2000) Bioavailability of 2, 4-dichlorophenol associated with soil water-soluble humic material. *Environ Sci Technol* 34(22): 4721–4726
- Shelton DR, Parkin TB (1989) A semiautomated instrument for measuring total and radiolabeled carbon dioxide evolution from soil. *J Environ Qual* 18:550–554
- Sims GK (2008) Stable isotope probing to investigate microbial function in soil. *Recent Res Dev Soil Sci* 2:64–85
- Sims GK, Cupples AM (1999) Factors controlling degradation of pesticides in soil. *Pestic Sci* 55:598–601
- Skidmore MW, Kirkpatrick D, Shaw D (1994) Influence of application methods on the degradation of permethrin in laboratory, soil aerobic metabolism studies. *Pestic Sci* 42:101–107
- Sumner ME, Miller WP (1996) Cation exchange capacity and exchange coefficients. In: Bartels JM, Bigham JM (eds) *Methods of soil analysis, part 4 chemical methods*. Soil Science Society of America, Madison, pp 1201–1230
- Taylor-Lovell S, Sims GK, Wax LM (2002) Effect of moisture, temperature, biological activity on degradation of isoxaflutole in soil. *J Agric Food Chem* 50:5626–5633
- Tor J, Xu C, Stucki JM, Wander M, Sims GK (2000) Trifluralin degradation under micro-biologically induced nitrate and Fe(III)-reducing conditions. *Environ Sci Technol* 34:3148–3152
- Vencill WK (2002) Herbicide handbook. Weed Science Society of America, Lawrence 493 p
- Walker A, Crawford DV (1970) Diffusion coefficients for two triazine herbicides in six soils. *Weed Res* 10:126–132
- Zaprasis A, Liu Y-J, Liu S-J, Drake HL, Horn MA (2010) Abundance of novel and diverse tfdA-like genes, encoding putative phenoxy alkanoic acid herbicide-degrading dioxygenases in soil. *Appl Environ Microbiol* 76(1):119–128
- Zhou J, Xia B, Huang H, Palumbo AV, Tiedje JM (2004) Microbial diversity and heterogeneity in sandy subsurface soils. *Appl Environ Microbiol* 70:1723–1734